

# Crop Nitrogen Uptake and Soil Phenols Accumulation under Continuous Rice Cropping in Arkansas

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Soil C stocks in the Grand Prairie region of eastern Arkansas have declined under the prevalent 2-yr rotation of rice (*Orzya sativa* L.)–soybean [*Glycine max* (L.) Merr.]. Continuous rice cropping could promote soil C sequestration, but in previous work continuous rice averaged 19% less grain yield than rice following soybean, apparently due to N deficiency. To further study N cycling, microplots were imbedded during the rice phase of a crop rotation field study in 2002 and 2004. Urea labeled with  $^{15}\text{N}$  was applied pre-flood, when all N fertilizer is conventionally applied. Crop biomass was often smaller with continuous rice than with rice following soybean (sampled both years) and rice following corn (*Zea mays* L.) (sampled only in 2004), although the difference varied by growth stage. Crop uptake of native  $^{14}\text{N}$ , presumably mineralized from soil organic matter, was inhibited with continuous rice in both years. This trend was clearest at harvest ( $P = 0.02$ ), when continuous rice averaged  $40 \text{ kg } ^{14}\text{N ha}^{-1}$  less uptake than rice in the two rotations. Fertilizer  $^{15}\text{N}$  averaged only 30% of total crop N and its uptake differed among cropping treatments only in 2002. At harvest, soil C with continuous rice cropping was enriched by 42% with syringyl phenols and by 83% with cinnamic phenols compared with the rotations. These enrichments appear unrelated to estimated input rates of lignin-derived phenols. Results support the hypothesis that continuous rice cropping promotes the binding of soil N by lignin-derived phenols, thereby inhibiting N mineralization and late-season crop growth. Similar observations were reported for tropical rice production, suggesting that the responsible soil processes might be common in continuous rice cropping.

Abbreviations: DAE, days after crop emergence.

The Grand Prairie is a productive rice region in eastern Arkansas (Fig. 1). Historically, this region has produced the highest rice grain yields in the state, and it provides a large proportion of the state's rice production. Paddy rice in this area is grown predominantly in a 2-yr rotation with soybean. The local agricultural soils are inherently low in soil C, with topsoils often having  $<10 \text{ g C kg}^{-1}$ . Rice–soybean cropping has further depleted soil C, to the detriment of soil physical properties (Scott and Wood, 1989). To rebuild soil C stocks, one option is to replace the rice–soybean rotation with a continuous rice monoculture, which has promoted soil C sequestration elsewhere (Cassman et al., 1998; Witt et al., 2000) due to slowed decomposition under the ex-

tended soil flood and probably also to increased input of root biomass compared with upland crops. Continuous rice cropping, however, has not been widespread in the Grand Prairie.

To evaluate a rice monoculture for grain yield sustainability and its effects on soil properties in the Grand Prairie, a long-term field experiment was begun in 1999 to compare rice monoculture with rice–soybean and other crop rotations at the University of Arkansas Rice Research and Extension Center in Stuttgart. Yield trends from 2000 to 2006 indicate a consistent grain yield gap: continuous rice has averaged 19% less grain yield than rice following either soybean (Anders et al., 2004, 2007) or corn (*Zea mays* L.) (unpublished data, 2007). Field monitoring indicated that the yield gap of continuous rice was not attributable to pest damage (insects, disease, or weeds), water deficiency, or crop establishment. Instead, the continuous rice crops showed initially vigorous growth, followed in mid-season by an abrupt slowing of growth compared with rice following soybean or corn. At physiological maturity of the 2002 and 2003 seasons, crop tissues for continuous rice had adequate concentrations of all measured nutrients (P, K, Ca, Mg, S, Zn, Fe, Mn, Cu, and B) except N (data not shown).

Similar symptoms were noted in the Philippines in long-term field experiments with two to three crops annually of irrigated lowland (paddy) transplanted rice for 20 or more years. Plant biomass increased at normal rates during the early growth stages, but in mid-season the leaf N concentration decreased to suboptimal levels (Cassman et al., 1995), followed by poor grain filling. These symptoms were associated with a long-term grain yield decline,

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which in turn was attributed largely to decreased availability of soil N (Cassman et al., 1995; Dobermann et al., 2000; Kropff et al., 2003), i.e., N that is not derived from fertilizer and is presumed to be mostly mineralized from soil organic matter. Most fertilizer N was applied as urea earlier in the growing season (preplant and panicle initiation growth stage); because fertilizer N does not persist in flooded soils, the crop became dependent on the soil N supply at later growth stages. The availability of soil N decreased despite a lack of decrease in the quantities of total soil N, a seeming paradox that focused research efforts on changes in the quality of soil N, i.e., its chemical nature.

Subsequent studies identified an accumulation of lignin-derived phenols as a key change in the chemical nature of the soil organic matter that accompanied long-term cropping of continuous rice (Olk et al., 1996, 1998, 2002). Lignin degrades slowly under flooded conditions. In laboratory studies, phenolic compounds chemically stabilize N into stable compounds (Verma et al., 1975; Thorn and Mikita, 1992; Thorn et al., 1996) that would be relatively less available to growing rice plants (Lynch and Lynch, 1958; Verma et al., 1975; Stevenson, 1994). No field evidence for this stabilization existed, though, until Schmidt-Rohr et al. (2004), using advanced nuclear magnetic resonance spectroscopy, detected anilide in a phenol-enriched humic fraction from a triple-cropped rice field experiment in the Philippines. In a related field study of double-cropped rice, the analysis of two soil humic fractions attributed an accumulation of lignin-derived phenols plus an accompanying inhibition of N mineralization to the conventional on-farm practice of anaerobic decomposition of crop residues, in contrast to aerobic decomposition (Olk et al., 2007).

The objective of this study was to determine whether the yield gap reported in the long-term field study of continuous rice cropping by Anders et al. (2004, 2007) is associated with an inhibited availability of soil N and accumulation in the soil of lignin-derived phenols. Soil N was distinguished from fertilizer N in plants by applying  $^{15}\text{N}$ -labeled fertilizer and assuming that the amount of  $^{15}\text{N}$  taken up into the plant represented fertilizer N, while the remaining plant N was derived from soil N.

## MATERIALS AND METHODS

### Site Description

The long-term field study was begun in 1999 at the Univ. of Arkansas Rice Research and Extension Center near Stuttgart (34°27' N, 91°24' W) on a Dewitt silt loam (fine, smectitic, thermic Typic Albaqualf), which is representative of the Grand Prairie soils. Initial soil properties included a pH range of 5.6 to 6.2 (1:1 soil/water) and mean contents of 8.4 g C kg<sup>-1</sup> soil and 0.6 g N kg<sup>-1</sup> soil as determined by automated combustion analysis. The study site had previously been fallowed due to a lack of irrigation capability.

### Field Treatments

The experimental design was described by Anders et al. (2004). Briefly, its main plots (76 by 12 m) were seven crop rotations, the subplots were two tillage treatments (conventional tillage and no-till), the sub-subplots were two fertilizer regimes, and the sub-sub-subplots (19 by 6 m) were two rice cultivars. The field had four replications. The seven crop rotations involve the major agronomic crops in Arkansas: rice, soybean, corn, and wheat (*Triticum aestivum* L.). For the purpose of this study, we selected sub-sub-subplots of continuous rice, rice-soybean, and rice-corn rotations, under conventional tillage, with the 'Wells' rice

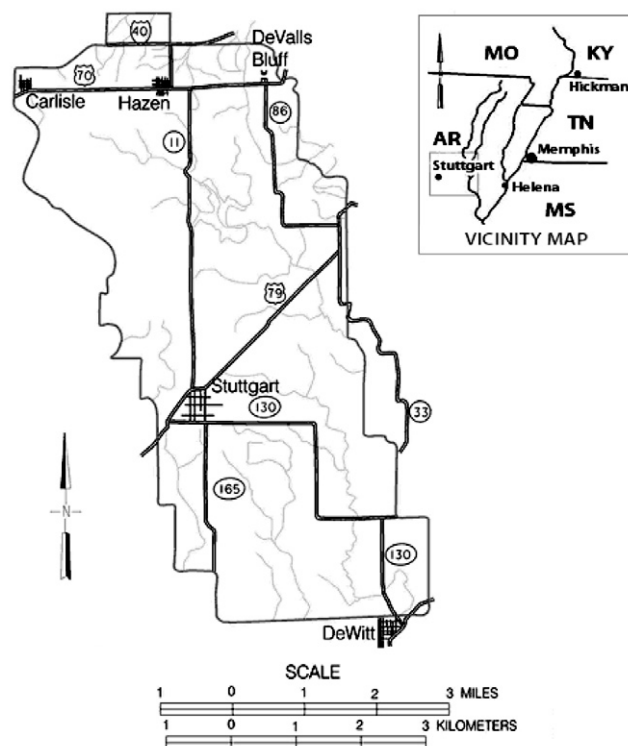


Fig. 1. The Grand Prairie region of eastern Arkansas.

cultivar and a high fertility regime. The experimental design of these selected treatments was a randomized complete block. All measurements were taken during the rice phase of each rotation in 2002 and 2004, although the rice-corn rotation was sampled only in 2004. The soybean cultivar was Asgrow 4702, and the corn cultivar was Dekalb C61-45.

The fertility treatment sampled here had rates intended for maximum grain yields, with 168 kg N ha<sup>-1</sup> (urea) applied with a hand spreader at the five-leaf stage of rice growth (17 June 2002 and 16 June 2004), 1 d before the field was flooded for the remainder of the growing season, following statewide recommendations. Microplots that would receive all their N fertilizer as a  $^{15}\text{N}$  label were covered by plywood during the hand spreading. For P and K, 26 kg P ha<sup>-1</sup> (triple superphosphate), and 75 kg K ha<sup>-1</sup> (KCl) were broadcast preplant with a hand spreader and then incorporated into the soil. Following the application of urea, a 5- to 10-cm-deep permanent flood was established and maintained on all plots until the rice reached physiological maturity.

Rice was drill-seeded into seedbeds at a rate of 100 kg seed ha<sup>-1</sup> with an Almaco (Almaco, Nevada, IA) no-till drill at a 19-cm row width. Sowing dates were 25 Apr. 2002 and 20 Apr. 2004 and seedling emergence was on 8 May 2002 and 28 Apr. 2004. Rice management closely followed the recommendations of the University of Arkansas Cooperative Extension Service regarding stand establishment, pest management, and irrigation management (Slaton, 2000). Crop residues were incorporated into the soil 1 to 2 mo following harvest through two disking operations. In the spring, the soil was tilled once by disking and once with a light field cultivator (triple-K), and finally it was land planed. Little information exists on the timing of residue decomposition during the autumn, winter, and spring seasons. The soil was wet and intermittently flooded during the cold fallow months due to rainfall.

### Nitrogen-15 Microplots

Four metal rings (60-cm diameter) were imbedded into each cultivar sub-sub-subplot to a depth of 10 cm. When the rice plants reached

the four- to five-leaf growth stage,  $^{15}\text{N}$ -enriched urea (5 atom%) was applied inside the rings at the same time and rate as the unlabeled urea was applied to each surrounding plot. The  $^{15}\text{N}$  fertilizer was applied by first dissolving it in  $\sim 4$  L of water in a metal sprinkling can, then sprinkling this solution onto the soil inside a ring, followed by sprinkling of another  $\sim 4$  L of water as a rinse of the metal can. To prevent movement of the soluble  $^{15}\text{N}$  out of the microplot, each microplot was flooded to a depth of 5 to 8 cm following application of the urea, and that depth was maintained for about 2 wk, presumably an adequate duration for soluble N to be removed from the floodwater through volatilization, soil adsorption, or plant uptake. After that time, rubber stoppers were removed from holes drilled into the ring and water from the surrounding plot was allowed to maintain water depth in the microplot.

## Soil and Plant Sampling

To characterize early-season conditions, soil samples were collected by hand probe from the 0- to 15-cm depth of each microplot 14 d after the application of N fertilizer, which was 53 d after plant emergence (DAE) in both years. Plant samples and accompanying soil samples were collected at three crop growth stages: (i) green ring (panicle initiation, about 83 DAE), (ii) 50% heading (flowering, about 103 DAE), and (iii) shortly before harvest. For each cultivar sub-sub-plot, two microplots were sampled at harvest to generate grain yield data and one microplot was sampled at each of the two earlier sampling times. In 2002, both harvest microplots were sampled immediately before the field was drained for harvest activities, at 133 DAE. In 2004, one microplot was sampled at the time of field drainage (127 DAE) and the other harvest microplot was sampled 3 wk later, during harvest of the main plot. Plant and soil properties were relatively similar between these two harvest samplings in 2004, so we report their means, as with the 2002 data. During sampling of a microplot, all the aboveground biomass was collected. Plant samples were dried at  $45^\circ\text{C}$ . The plant samples collected at harvest were threshed to separate grains from the remaining plant material to determine grain yield, and in 2004 the stems were also separated from the leaves. Dried plant samples were weighed and finely ground. Soil samples were passed through an 8-mm mesh and large roots were removed. Soil samples were air dried at ambient temperature and finely ground.

The N concentration in the most recently developed leaf, or “y” leaf, has been presumed to represent the current N status of the plant (Thenabadu, 1972). During the 2002 growing season, the “y” leaf was periodically sampled for plants in the main plots (but outside the microplots) of the continuous rice and rice-soybean treatments. Leaves were combined into composite samples for each rotation, dried by microwaving, and ground.

## Laboratory Analyses

Dried soil samples and subsamples of all dried plant samples that were taken from the microplots were analyzed for  $^{15}\text{N}$  and total N content by continuous-flow isotope ratio mass spectroscopy, using a Fisons NA 1500 NC Series 2 elemental analyzer (Fisons Instruments, Beverly, MA) coupled to a Finnigan Delta A mass spectrometer (Thermo Electron Corp., San Jose, CA). Crop uptake of fertilizer N was calculated from the  $^{15}\text{N}$  concentrations of plant samples and the atomic percentage of the  $^{15}\text{N}$  urea. Total soil C was measured by automated combustion analysis on the same elemental analyzer during an initial step in the analysis for soil  $^{15}\text{N}$  and total N contents. The amount of inorganic soil C was found to be negligible (mean  $0.2\text{ g kg}^{-1}$ ) in the 2004 soils by a modified pressure-calimeter method (Sherrod et al., 2002), so the total soil C of the 2002 soil samples was presumed to adequately represent soil organic C.

Soil concentrations of lignin-derived phenols were determined by CuO oxidation through a procedure modified from Hedges and Mann (1979). Samples were placed in pressure bombs together with NaOH and CuO as an oxidizing agent. The bombs were purged with Ar gas, sealed, and heated for 3 h at  $150^\circ\text{C}$ . The phenols were extracted from the solution through repeated ether washes and centrifugations, then filtered and derivatized through silylation with bis(trimethylsilyl)trifluoroacetamide. Samples were analyzed by gas chromatography for their concentrations of ferulic acid and *p*-hydroxycoumaric acid (together called cinnamic acids), and aldehyde, ketone, and acid forms of syringyl, vanillyl, and *p*-hydroxybenzoic phenols. Soil *p*-hydroxybenzoic phenols arise from multiple plant and microbial sources and did not show treatment effects, so their concentrations are not reported here. Phenol concentrations are reported on the basis of soil organic C.

To determine the phenolic composition of crop tissues, root and straw samples were collected from unrelated field studies of rice, soybean, and corn. Crop cultivars were MTL-250 (rice), Asgrow AG2203 (soybean), and Pioneer 34B24 BT (corn). They were analyzed by CuO oxidation following the procedure described above.

To measure the N concentration of the “y” leaves, the sampled leaves were combined with Orange-G dye and measured for light absorption at 482 nm, and these values were converted into N concentration as described by Hafez and Mikkelsen (1981).

## Statistical Analysis

Treatment effects were statistically evaluated for grain yield; crop biomass; plant contents of  $^{15}\text{N}$ ,  $^{14}\text{N}$ , and total N; and soil contents of vanillyl, syringyl, and cinnamic phenols. Statistical significance of the treatment effect was established through analysis of variance using a SAS general linear model program that was appropriate to the randomized complete block design of the sampled field plots. In 2004, the cropping treatment effect was statistically evaluated by both (i) Duncan’s multiple-range test, and (ii) comparison of continuous rice with the group mean of rice-soybean and rice-corn, where the level of significance was based on a linear single degree of freedom contrast. This second approach is possible because the field experiment was established based on preexisting knowledge that the continuous rice rotation differed from all other cropping systems in its effects on soil properties (Littell et al., 2002).

## RESULTS

### Rice Grain Yield and Biomass

In 2002, the grain yield in the  $^{15}\text{N}$ -amended microplots was less ( $P = 0.033$ ) for continuous rice ( $6.8\text{ Mg ha}^{-1}$ , 12% moisture basis) than for rice following soybean ( $9.3\text{ Mg ha}^{-1}$ ). In 2004, the grain yield of continuous rice was  $7.2\text{ Mg ha}^{-1}$ , which was less ( $P = 0.036$ ) than the mean of rice following either soybean ( $8.1\text{ Mg ha}^{-1}$ ) or corn ( $9.1\text{ Mg ha}^{-1}$ ). Averaged across both years and all rotations, continuous rice yielded 19% less grain than the other two rotations, similar to treatment differences measured in previous years for the main plots (Anders et al., 2004, 2007).

Compared with these grain yield trends, the effect of crop rotation on the total aboveground biomass was less consistent in both years. In 2002, rice plant biomass became clearly different between continuous rice and the larger plants in the rice-soybean rotation only after the 50% heading stage (Fig. 2, left). In 2004, the biomass of the continuous rice was significantly less than for the other two rotations solely at the green ring growth stage, although it was numerically less at both heading and harvest stages (Fig. 2, right). Averaged across all six sampling times, plant bio-

mass was 11% less for continuous rice than for rice following soybean.

## Crop Nitrogen Uptake

During the 2002 season, the N content of the “y” leaf differed little between the continuous rice and rice–soybean treatments through 25 July, shortly before the green ring growth stage (Fig. 3). With continuing crop growth, leaf N content decreased for both treatments, but more so for continuous rice. This mid-season decrease coincided roughly with visual symptoms of slowing growth in the continuous rice treatment, consistent with previous years (data not shown).

This crop rotation effect on the N content of the “y” leaf was confirmed by trends in total crop N uptake. In 2002 (Fig. 4, left), crop N uptake did not differ significantly ( $P = 0.28$ ) at the green ring growth stage, although it was numerically less for continuous rice than rice following soybean. For the remainder of this growing season, crop N uptake increased slowly in continuous rice while it increased more rapidly in rice following soybean. This treatment difference was significant ( $P = 0.02$ ) at harvest. Its lack of significance ( $P = 0.40$ ) at the heading stage was caused by high variability among field replicates, probably due to uneven crop stands. Shortly after sowing, the weather remained cool and wet for a considerable amount of time, causing seed rotting and requiring reseeded in portions of the field. In 2004 (Fig. 4, right), N uptake increased at a slower rate for continuous rice than for the other two rotations, more clearly in later growth stages than at the green ring stage, which is consistent with the results for 2002. The difference between continuous rice and the mean of the other two rotations was significant ( $P < 0.05$ ) at two of the three sampling times. Crop N uptake for rice following corn was comparable to that of rice following soybean.

Crop N was partitioned into fertilizer N and soil N based on the assumption that all crop content of  $^{15}\text{N}$  represented fertilizer N. Following the single application of all N fertilizer at 39 DAE, crop uptake of fertilizer N appeared to have essentially ceased by the first plant sampling at green ring (83 DAE) for all crop rotations in both 2002 (Fig. 5, left) and 2004 (Fig. 6, left), in general agreement with earlier results by Patrick and Reddy (1976), Wilson et al. (1989, 1990), and Norman et al. (1992). In contrast, crop uptake of  $^{14}\text{N}$ , presumably soil N, continued until the harvest sampling in both years in all treatments (Fig. 5, right; Fig. 6, right).

In 2002 (Fig. 5), fertilizer N uptake differed significantly between continuous rice and rice following soybean only at harvest (133 DAE). A numeric difference enlarged progressively during the growing season because fertilizer N uptake by continuous rice steadily decreased. This decrease was not reproduced in 2004 (Fig. 6), although it has been reported in previous rice studies involving  $^{15}\text{N}$ -labeled fertilizer, as reviewed by Norman et al. (1992), who attributed the decrease to multiple potential pathways for  $^{15}\text{N}$  loss from the plant. In 2004, fertilizer N uptake was unaffected by crop rotation.

In each year, soil N uptake was numerically less for continuous rice than for rice following soybean at all three sampling times, and in 2004 rice following corn behaved similarly to rice

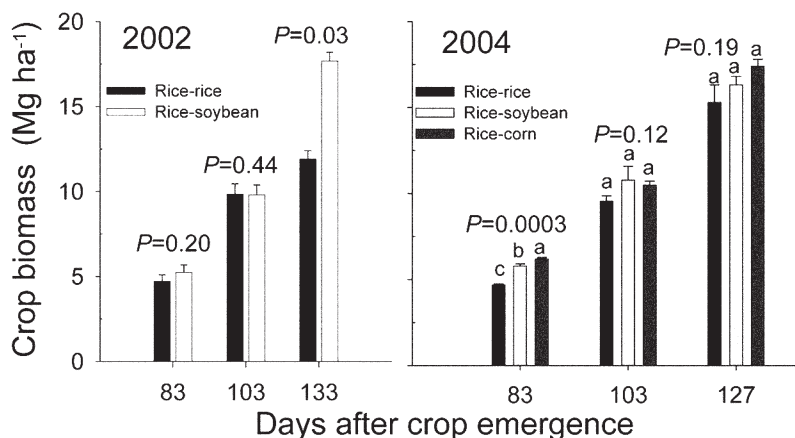


Fig. 2. Total aboveground crop biomass at three sampling times in the 2002 and 2004 seasons for two and three cropping treatments, respectively. Crop growth stages at sampling times were green ring (83 d after crop emergence [DAE]), 50% heading (103 DAE), and shortly before harvest (133 or 127 DAE). Standard error bars are shown for each crop treatment. Levels of significance are shown for the comparison of continuous rice with rice–soybean (2002) or the comparison of continuous rice with the group mean of rice–soybean and rice–corn (2004) where the level of significance was based on a linear single degree of freedom contrast. For each sampling time in 2004, cropping treatments shown with different letters are significantly different ( $P < 0.05$ ) as determined by Duncan’s multiple-range test.

following soybean. The inhibition of soil N uptake with continuous rice became more evident at the later sampling times; at harvest it was statistically significant ( $P = 0.02$ ) in both years. In 2002, high variability among field replicates precluded its significance at the heading stage.

Averaged across both years, crop uptake of soil N at harvest was  $40 \text{ kg N ha}^{-1}$  less for continuous rice than for rice following either soybean or corn. Assuming a physiological efficiency of  $50 \text{ kg grain kg}^{-1} \text{ N uptake}$  (Yoshida, 1981), this N loss translates into a yield loss of  $2.0 \text{ Mg ha}^{-1}$ , which is equivalent to the yield gap between the continuous rice treatment and the rotations with corn or soybean. Overall, the increase in rice uptake of N following the soybean or corn rotation (Fig. 4) was mostly due to an improved uptake of soil N, not fertilizer N.

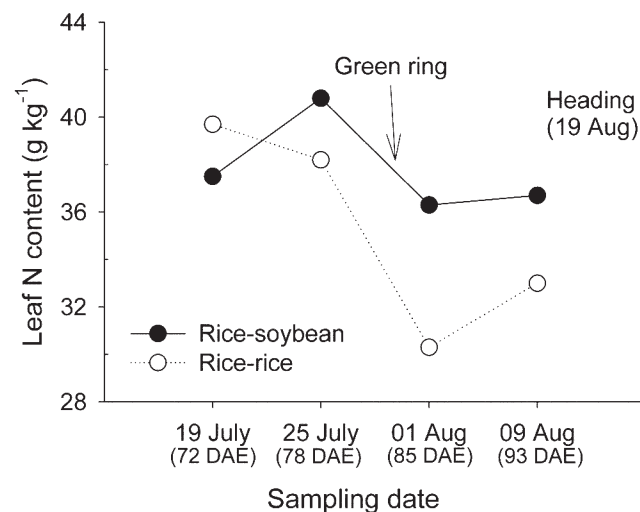


Fig. 3. Leaf N concentration for the “y” leaf of rice plants in two cropping treatments during the 2002 season. Sampling dates are described by their number of days after crop emergence (DAE).

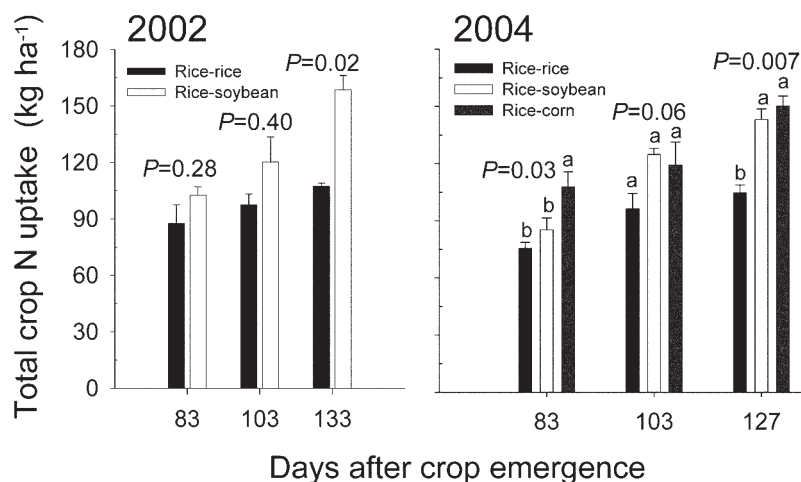


Fig. 4. Total crop N uptake at three sampling times in the 2002 and 2004 seasons for two and three cropping treatments, respectively. Crop growth stages at sampling times were green ring (83 d after crop emergence [DAE]), 50% heading (103 DAE), and shortly before harvest (133 or 127 DAE). Standard error bars are shown for each crop treatment. Levels of significance are shown for the comparison of continuous rice with rice-soybean (2002) or the comparison of continuous rice with the group mean of rice-soybean and rice-corn (2004) where the level of significance was based on a linear single degree of freedom contrast. For each sampling time in 2004, cropping treatments shown with different letters are significantly different ( $P < 0.05$ ) as determined by Duncan's multiple-range test.

### Soil Nitrogen and Nitrogen-15 Contents

The soil content of total N varied from 507 to 770 mg kg<sup>-1</sup>, and differences among crop treatments were inconsistent across sampling times in both 2002 and 2004. Therefore, total soil N was not correlated with crop N uptake (data not shown), as was previously reported for Asian paddy rice soils by Cassman et al. (1996, 1998). Seasonal shifts during the growing season for each treatment were also inconsistent in both years. Probably these reflect random error magnified by the low levels of soil N. High soil N contents at the 2002 harvest sampling were accompanied by high contents of soil C (data not shown), suggesting an errant sampling depth.

The soil content of fertilizer N, based on the <sup>15</sup>N concentration, ranged in 2002 from 17.8 to 51.2 kg ha<sup>-1</sup> and in 2004 from 6.6 to 18.5 kg ha<sup>-1</sup>. Similar to total soil N, soil fertilizer N showed high variability among field replicates, probably also

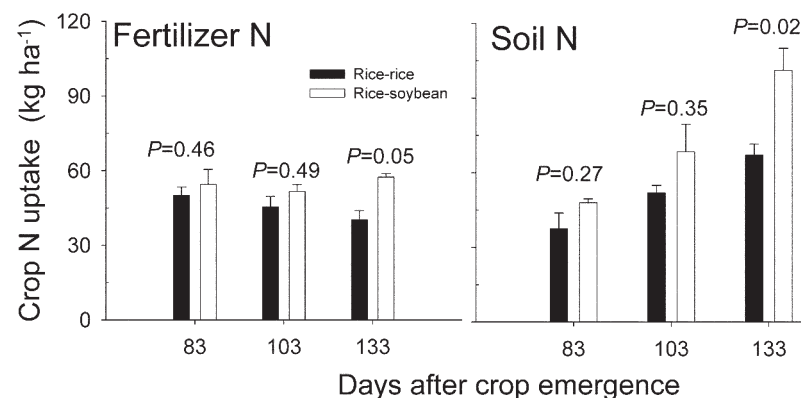


Fig. 5. Crop uptake in 2002 of fertilizer N and soil N for two cropping treatments. Crop growth stages at sampling times were green ring [83 d after crop emergence (DAE)], 50% heading (103 DAE), and shortly before harvest (133 DAE). Standard error bars are shown for each cropping treatment. Levels of significance are shown for the comparison of continuous rice with rice-soybean.

reflecting random errors among these low values and errant sampling depths. No pattern was apparent among crop growth stages or crop rotations that held true for both years other than high values at the harvest sampling as noted for total soil N.

### Lignin-Derived Soil Phenols

Relative abundances in the soil of acid, aldehyde, and ketone forms for the vanillyl and syringyl groups were fairly stable among cropping treatments, as indicated by the ratios of acid/aldehyde forms (Table 1), so they were pooled within each group. Ferulic acid and *p*-hydroxycoumaric acid were summed and are represented as cinnamic acids. For the continuous rice and the rice-soybean treatments, these three phenol groups were more enriched per kilogram soil C at the harvest sampling than at 53 DAE in both 2002 (Fig. 7) and 2004 (Fig. 8). Because the phenol concentrations are expressed per unit soil C, this seasonal shift might indicate relative phenol enrichment as other, more labile C compounds in the soil were preferentially degraded. Notably, soil phenol levels for rice following corn in 2004 were not clearly greater at harvest than at 53 DAE.

Cinnamic phenols were, in most cases, more enriched in the continuous rice soil than in the soils of the two rotations, especially at the harvest sampling ( $P < 0.01$ ), when they were 83% more abundant, averaged across years. Syringyl phenols were also more enriched in continuous rice, being 42% more abundant ( $P < 0.01$ ) than in the two rotations, averaged across both harvests. In contrast, vanillyl phenols were numerically more abundant in rice following soybean than in the other two crop treatments, although the level of significance varied. The soil vanillyl difference might reflect plant biochemical compositions: CuO analysis of other plant cultivars from other locations found that soybean stem was two and five times more enriched in vanillyl phenols than corn stem and rice stem, respectively, while cinnamic phenols were scarcely present in soybean stem (Table 2). Corn stem had 74 to 179% more phenols than did rice stem for the three phenol groups. Crop root biomass was not measured in this study, but roots are also likely to contribute substantial amounts of phenols. Their phenol concentrations were closely correlated ( $r = 0.97$ ) with the stem concentrations. We did not measure phenol concentrations in other plant components, such as corn cobs and young leaves, but leaves should have considerably smaller phenol concentrations than shoots and roots.

Soil phenol contents can reflect not only plant phenol concentrations but also the biomass of incorporated crop residues. The biomass of crop stem was calculated from multiyear means in this field experiment. If the phenol concentrations that we measured for crop cultivars from other locations are representative of the cultivars grown in the field, rice stem biomass would have exceeded corn stem biomass sufficiently to counteract the greater phenol enrichment of corn stem (Table 2) so that the incorporation rate into the soil of stem-derived phenols would have been comparable for

the two crops, and both would have been substantially greater than that of soybean stem. Hence this partial accounting of total incorporated phenols fails to explain the soil phenol accumulation with continuous rice compared with the two rotations.

The ratio of acid/aldehyde forms for vanillyl or syringyl phenols has often been interpreted as an index for the degree of microbially induced oxidation (Ertel and Hedges, 1984). As described above, this ratio did not differ consistently between the continuous rice treatment and the two rotations for either the vanillyl or syringyl phenols (Table 1). In most cases, this ratio was smaller at harvest than at 53 DAE for both the vanillyl and syringyl phenols. This seasonal shift was more significant for the vanillyl phenols than the syringyl phenols, and it was more significant for rice following soybean than for the other two crop treatments.

## DISCUSSION

The objective of this study was to elucidate the soil processes responsible for the yield gap that has accompanied continuous rice cropping in the Grand Prairie. This monoculture could enable increased C inputs to the soil, potentially helping reverse the observed degradation of soil physical properties that has occurred under rice–soybean cropping. Unless this yield gap is resolved, though, adoption of continuous rice cropping will be inhibited.

The yield gap appears to result from decreased N status in the plant that develops at midseason growth stages (Fig. 3). No other yield constraint has been apparent in this long-term experiment: there has been no evidence for a plant-based inhibition of biomass growth, such as pest damage, toxicity, or deficiency of any nutrient other than N.

Rice N status decreased in midseason after the crop uptake of fertilizer N had slowed, several weeks after the sole fertilizer application and consequently at a time when crop N uptake had become dependent on soil-derived N, presumably mineralized from soil organic matter. Rice uptake of soil N differed slightly among the crop rotations at the first sampling time (green ring), but its inhibition with continuous rice cropping became clearest during later growth stages in both 2002 and 2004. This timing is consistent with visual observations of impaired crop growth that developed with continuous rice cropping only in later growth stages. Therefore distinguishing between fertilizer N ( $^{15}\text{N}$ ) and soil N ( $^{14}\text{N}$ ) was critical to this study, as was the case in studies describing yield decline in Philippine long-term experiments of monoculture rice (Cassman et al., 1995; Dobermann et al., 2000).

We gained further evidence for an N cause of the yield gap during long-term on-farm production of continuous rice: historical grain yields could be maintained only through multiple

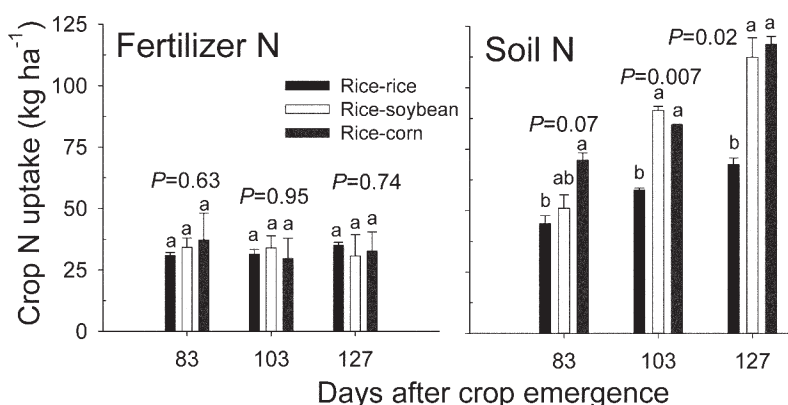


Fig. 6. Crop uptake in 2004 of fertilizer N and soil N for three cropping treatments. Crop growth stages at sampling times were green ring (83 d after crop emergence [DAE]), 50% heading (103 DAE), and shortly before harvest (127 DAE). Standard error bars are shown for each crop treatment. Levels of significance are shown for the comparison of continuous rice with the group mean of rice–soybean and rice–corn where the level of significance was based on a linear single degree of freedom contrast. For each sampling time, cropping treatments shown with different letters are significantly different ( $P < 0.05$ ) as determined by Duncan's multiple-range test.

applications of N fertilizer (personal observation). Similarly, the yield decline for monoculture rice in the Philippines was reversed largely through multiple midseason applications of N fertilizer that compensated for the loss of soil N availability (Cassman et al., 1995; Dobermann et al., 2000; Kropff et al., 2003). These common findings suggest that continuous rice cropping heightens the need for additional applications of N fertilizer to maintain N availability throughout the growing season compared with the rice–soybean rotation. Alternatively, crop uptake of soil N might be improved through aerobic decomposition of incorporated rice residues, which in one Philippine study improved in-season mineralization of humic N and limited the accumulation of lignin-derived phenols (Olk et al., 2007).

As in the Philippine studies, the inhibition of soil N cycling with continuous rice cropping in this study was associated with a soil accumulation of lignin-derived phenols. Analysis of a phenol-rich humic fraction from the continuous rice soil by advanced nuclear magnetic resonance spectroscopy demonstrated the occurrence of anilide N (Olk et al., 2006), although the quantity and agronomic significance of this chemically bound N

Table 1. Ratios of acid to aldehyde concentrations for soil vanillyl and syringyl phenols at two sampling times during rice phases of two (2002) or three (2004) crop rotations: rice–soybean, rice–rice, and rice–corn. The sampling times were (i) 14 d after N fertilizer application (53 d after crop emergence [DAE]), and (ii) shortly before harvest (133 or 127 DAE for 2002 and 2004, respectively). The indicated level of significance ( $P$ ) is for the difference between the two sampling times in each year. Standard errors are in parentheses.

Crop rotation	Acid/aldehyde concentration ratio					
	2002			2004		
	53 DAE	Harvest	$P$ level	53 DAE	Harvest	$P$ level
<u>Vanillyl phenols</u>						
Rice–rice	0.48 (0.02)	0.44 (0.03)	0.048	0.72 (0.02)	0.51 (0.02)	0.018
Rice–soybean	0.48 (0.02)	0.41 (0.01)	0.012	0.68 (0.02)	0.43 (0.03)	0.006
Rice–corn	–	–	–	0.66 (0.06)	0.58 (0.05)	0.087
<u>Syringyl phenols</u>						
Rice–rice	0.43 (0.01)	0.44 (0.02)	0.297	0.55 (0.03)	0.47 (0.02)	0.122
Rice–soybean	0.46 (0.01)	0.41 (0.02)	0.100	0.60 (0.02)	0.45 (0.03)	0.016
Rice–corn	–	–	–	0.54 (0.03)	0.52 (0.03)	0.037

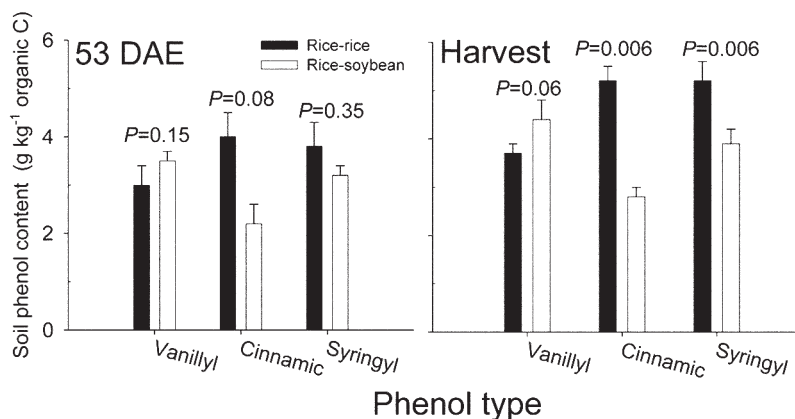


Fig. 7. Soil content of three types of lignin-derived phenols for two cropping treatments in 2002, 53 d after crop emergence (DAE) and shortly before harvest. Standard error bars are shown for each crop treatment. Levels of significance are shown for the comparison of continuous rice with rice-soybean.

form was not addressed. These results are all consistent with but do not prove our hypothesis that phenolic binding of soil organic N inhibited crop uptake of soil N at later growth stages and hence caused a yield reduction. An alternative explanation for this assemblage of soil and plant observations is not apparent.

In general, our soil and plant observations closely paralleled those made in the Philippine studies. One difference was the immediate appearance of the yield gap in the first year of the Arkansas field experiment (Anders et al., 2004), while the Philippine yield decline began only after a few years of multiple annual cropping (Cassman et al., 1995). This difference might reflect background levels of soil organic matter: the much lower organic matter contents of the Arkansas soils could amplify the inhibitory effect of N stabilization on soil N cycling, while the rich (20–30 g C kg<sup>-1</sup> soil) Philippine soils seem better able to release ample amounts of soil N for several growing seasons even if soil N were increasingly stabilized through phenolic binding.

The striking similarity in crop symptoms between the Philippines and Arkansas studies initially suggests that the inhibited soil N cycling and its underlying processes are general

traits of rice monoculture. Olk et al. (2007), however, attributed the soil accumulation of lignin-derived phenols under continuous rice cropping to anaerobic decomposition of crop residues, and they found that the phenol enrichment in a young humic fraction was greater at higher grain yield levels, where greater amounts of crop residues would be incorporated into the soil. Hence even if the soil processes responsible for the inhibition of N cycling were general traits of monoculture rice cropping, the strength of their expression and effect on crop growth would depend on local conditions, including crop biomass level, soil aeration at the time of residue incorporation, synchrony of N fertilizer application with crop N demand, and whether yield levels are constrained simultaneously by other factors unrelated to N. We hypothesize that an accumulation of lignin-derived phenols blocks only a portion of the soil N supply, which would depress yields only when crop growth is constrained by the N supply.

The mechanisms by which lignin-derived phenols inhibit soil N cycling might be more complex than merely strong covalent binding between monomeric amino compounds and phenols to form anilide-like structures. Alternative binding modes have been proposed between organic compounds and phenols or the related quinones (Theis, 1945; Thomson, 1964) that would probably result in weaker bonds and shorter durations of binding compared with anilide. Future research should investigate whether phenolic stabilization might primarily act to delay soil N mineralization to a time when rice plants do not take up N (at harvest or during the subsequent fallow), rather than prevent N mineralization altogether.

The phenol results raise some questions beyond their potential role in N stabilization. The abundance of cinnamic phenols in the continuous rice soil compared with the rice-soybean soil was expected, based on their known enrichment in grasses such as rice compared with dicotyledonous plants such as soybean (Chen, 1992). Corn is also a grass, and soil levels of all three phenol groups for rice following corn at the early sampling (53 DAE) in 2004 (Fig. 8, left) were nearly as high as those of continuous rice, perhaps reflecting comparable rates of phenol input (Table 2). Yet soil levels of lignin-derived phenols for rice following corn did not increase from the early-season sampling to the harvest sampling (Fig. 8, right), in contrast to continuous rice and even the rice-soybean rotation. Apparently lignin-derived phenols from corn were decomposed at a faster rate during the rice season than were those of rice and soybean, although all three treatments were in the rice phase. An explanation is currently lacking; speculative reasons include divergent soil microbial activity or weaker binding of corn phenols in either crop residues or soil organic matter. Finally, we cannot explain the downward seasonal shift in the acid/aldehyde ratios for both vanillyl and syringyl phenols (Table 1). Microbial activity during the warm summer months of the rice growing season should promote oxidation of soil phenols, causing the ratios to increase. Possible explanations

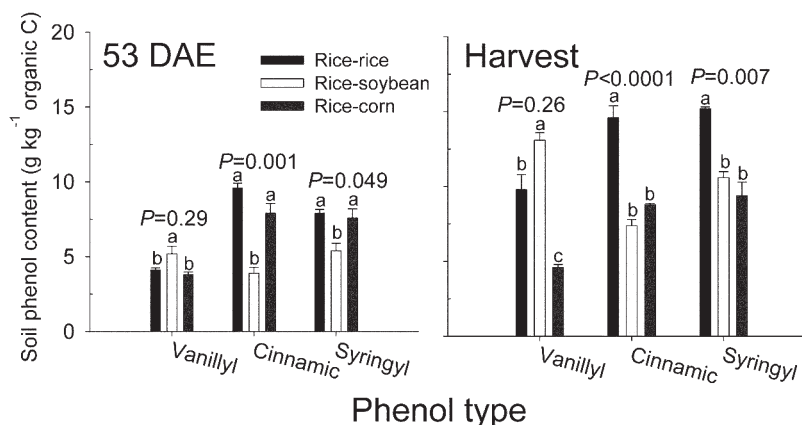


Fig. 8. Soil content of three types of lignin-derived phenols for three cropping treatments in 2004, 53 d after crop emergence (DAE) and shortly before harvest. Standard error bars are shown for each crop treatment. Levels of significance are shown for the comparison of continuous rice with the group mean of rice-soybean and rice-corn where the level of significance was based on a linear single degree of freedom contrast. For each sampling time, cropping treatments shown with different letters are significantly different ( $P < 0.05$ ) as determined by Duncan's multiple-range test.

include (i) loss of acidic phenols through solubilization into the floodwaters, and (ii) an increase in extractable aldehyde phenols during the growing season through root sloughing or initial breakdown of crop residues.

In this study, fertilizer N accounted for only 30% of total crop N uptake, averaged across all cropping treatments at harvest. Averaged across all sampling times, the amount of fertilizer N in the crop was 30% of the total fertilizer amount applied in 2002, and only 19% in 2004. By comparison, Cassman et al. (1998) reported a range of 36 to 39% for fertilizer uptake efficiency in two studies in tropical Asia, and Norman et al. (1989, 1992) reviewed results from several U.S. studies that ranged from 25 to >50%. Nitrogen uptake efficiencies can vary among studies depending on the rates and timing of fertilizer application and whether the <sup>15</sup>N-amended plants are managed similarly to the surrounding crop.

## CONCLUSIONS

In the Grand Prairie of eastern Arkansas, a grain yield gap that occurs with continuous rice cropping was associated with decreased crop uptake of soil N in the mid- and late-season growth stages, compared with the conventional rice–soybean rotation and rice–corn rotation. This decrease was associated with an accumulation in the soil of lignin-derived phenols, suggesting chemical stabilization of soil N by phenols. These results are consistent with earlier work with rice monoculture cropping in the Philippines. This work suggests that grain yields in continuous rice can be improved through multiple applications of N fertilizer to maintain N availability at later crop growth stages. An alternative solution might be aerobic decomposition of incorporated crop residues to improve the availability of soil N.

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**Table 2. Estimated input into soil of lignin-derived phenols from crop stems Phenols are pooled into vanillyl, syringyl, and cinnamic phenols.**

Crop	Concentration of phenols in stem†	Stem biomass‡	Total phenol input
	g kg <sup>-1</sup>	kg ha <sup>-1</sup>	
	<u>Vanillyl</u>		
Rice	23	6358	146
Soybean	118	1440	170
Corn	47	2995	141
	<u>Syringyl</u>		
Rice	44	6358	278
Soybean	106	1440	153
Corn	123	2995	368
	<u>Cinnamic</u>		
Rice	90	6358	572
Soybean	2	1440	3
Corn	157	2995	470

† Values were determined on crop cultivars not grown in this field study.

‡ Mean value of multiple growing seasons in this field experiment.

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